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TITLE: Genomic and Expression Profiling of Benign and Malignant
Nerve Sheath Tumors in Neurofibromatosis Patients

PRINCIPAL INVESTIGATOR: Matt van de Rijn, M.D., Ph.D.
Torsten Nielsen, M.D., Ph.D.
Brian Rubin, M.D., Ph.D.

CONTRACTING ORGANIZATION: Leland Stanford Junior University
Stanford, California 94305-4125

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13. ABSTRACT (Maximum 200 Words) The goal of this project is to identify genes involved in the malignant transformation of neurofibromas to malignant peripheral nerve sheath tumors using expression profiling and array-based comparative genomic hybridization. The significance of the genes will be validated on much larger numbers of cases using antibodies and in situ hybridization probes on tissue microarrays (TMAs). Genes will be further studied in in vitro experiments using cell lines from nerve sheath tumors. While the grant starting date was on May 1, 2003 authorization to work with human subjects was not obtained until April 1, 2004. Therefore this "annual report" will only describe the actual work performed in April 2004. Nevertheless the following progress has been made in the past year: 1. The number of cases of nerve sheath tumors available will be more than sufficient to perform the first aim of this study. 2. We have started to run expression profiling and gene microarrays on a number of nerve sheath tumors and since April 1, 2004 have analyzed six malignant peripheral nerve sheath tumors and five schwannomas. 3. We have gained much experience with in situ hybridization on TMAs. This experience will enormously benefit this project.				
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INTRODUCTION

Malignant transformation of benign neurofibromas is a life threatening complication in patients with neurofibromatosis. The goal of this study is to identify markers that may help the clinician determine whether a malignant transformation of a benign neurofibroma is actually in progress in a patient. We will accomplish this through a large scale genome-wide expression profiling study on benign lesions and malignant lesions originating in the nerve sheath to find genes that can function as markers of this malignant transformation. In this genome-wide search we will also use comparative genomic hybridization. In this technique, the presence of gene amplifications or deletions in tumor samples is determined. Subsequently we will validate and extend these findings on tissue microarrays (TMAs) containing very large numbers of these tumors using paraffin-embedded, formalin-fixed material. These TMAs will be examined by immuno-histochemistry with existing or de novo generated antisera or by in situ hybridization for the genes of interest.

The ultimate goal is to find markers that will help us distinguish benign from malignant nerve sheath tumors and that ultimately may lead to a serological marker to follow disease progression as well. In addition it can be expected that we may find several novel potential therapeutic targets for the treatment of malignant nerve sheath tumors.

BODY

The initiation of this project was delayed by eleven months by issues regarding the authorization to work with human subject material. However, it should be stressed that once I got into direct contact with Dr. Inese Beitins, the process actually went very quickly. I wish that I had known of her existence much earlier. On February 26 I sent her a letter after having been advised by her through a telephone conversation. Subsequently we had the approval to proceed by April 1. I mention this in detail because I can imagine that these issues might occur with other grants as well and I believe that an unnecessary delay of

ten months could have been prevented had I known of Dr. Beitins' existence so that I could have contacted her sooner.

Despite the delay incurred we have been able to make significant progress. In the past year I have inventoried the number of nerve sheath tumors currently available to me and have determined that these are sufficient to perform the initial phase of the study using gene microarrays to perform expression profiling and comparative genomic hybridization. As of April 1st, 2004, we also have started to perform these studies as described in the section below. Importantly, in parallel projects performed in my laboratory we have developed an enormous amount of experience with in situ hybridization on tissue microarrays and the experience gained in this area will be extremely valuable once we identify genes of interest in the nerve sheath tumor project.

Specific aim 1: "Genome-wide search for genes in nerve sheath tumors"

Initial expression profiling experiments using 40,000 elements cDNA microarrays were performed on six malignant peripheral nerve sheath tumors, five schwannomas and six synovial sarcomas (Figure 1). Gene filtering was then performed to remove genes that were poorly measured and to remove those genes that show no significant variation across the samples. Specifically, using selection criteria for signal over-background measurement of quality of the signal intensity 34,000 genes passed the filters. The subsequent filtering that selected for genes that varied at least fourfold in at least two of the arrays removed approximately 30,000 genes. In the final filtering step we removed genes that were not well measured in at least 80% of the data. As a result of these rather stringent filtering criteria, 1,920 genes were selected for the hierarchical clustering. The gene selection as described above is just one of many gene selections that we will perform on this data set and that we will continue to perform as the actual data set continues to grow through additions of more nerve sheath tumor specimens and other sarcomas. Unsupervised hierarchical clustering was performed on the 1,920 gene data set and as shown in Figure 1 all synovial sarcomas clustered on a branch distant from the other tumors. Likewise all schwannomas clustered on a separate branch. The malignant peripheral nerve sheath tumors showed a distribution over three branches, one of which (branch two) contained a majority (four cases) of the samples while the other two cases (STT3990 and STT3994) were on separate branches of the branch that contained that contained all schwannomas. These

findings show that malignant peripheral nerve sheath tumors are a heterogeneous group of tumors and they emphasize the need for detailed comparison with histologic findings and gene array studies. In the collaboration between myself and Drs. John Goldblum, Brian Rubin and Torsten Nielsen we have sufficient surgical pathology expertise to guarantee this.

Of course this data set is still quite small and much smaller than the one that we hope to obtain in the next 1 to 1 1/2 years. Nevertheless as an example of this study we have performed a SAM analysis to determine the genes that are most significant in the separation of malignant peripheral nerve sheath tumors from the other lesions. An example of this SAM analysis is shown in Table 1 where the 112 most significant genes that determined separation from MPNST from the other tumors are shown. Interestingly for several of these genes including CTHRC1, EGFR, we have already generated in situ hybridization probes in parallel projects on fibromatosis studies. These probes will act as validation tools of gene array data using tissue microarrays.

Specific aim 2: "Validation of candidate genes"

An important development in my laboratory is that we have become very familiar with the generation and use of in situ hybridization probes on our TMAs. In situ hybridization on formalin fixed, paraffin embedded material has long been problematic. We have started using a method (published by others) that incorporates tyramide amplification of signal to perform a non-radioactive in situ hybridization on our TMAs. In the appendix I have included a preprint of a paper, currently in press in the American Journal of Pathology, where we describe a novel marker in GIST tumors and where examples of this technique can be seen. I expect that incorporation of this technique in the nerve sheath tumor project will markedly increase the number of candidate genes that we can examine on TMAs. This is because antiserum production (as proposed in the original grant application) is a very long-term proposal with a turnaround of at least 4-6 months. In contrast, in situ hybridization probes can be generated within 10 days. We still expect to generate significant numbers of conventional antisera as they will allow us to study the proteins rather than the mRNA for interesting genes but we also expect that in situ hybridization will be used as a first determination for the significance of genes identified by expression profiling or comparative genomic hybridization.

KEY RESEARCH ACCOMPLISHMENTS

1 Inventory of nerve sheath tumors available at Stanford.

This has been completed and the number of cases will be sufficient to perform the initial phase of the study.

2 Initiation of gene array studies.

See section above.

3 Development of in situ hybridization.

We have generated a lot of experience with non-radioactive in situ hybridization on TMAs. To date we have generated 52 probes. This will tremendously speed up our ability to examine large numbers of genes on TMAs.

REPORTABLE OUTCOMES

No reportable outcomes are available. The study has really only has been started for the last month.

CONCLUSIONS

The data shown above forms an important proof of principle that we can perform these high volume experiments on the available samples. The RNA quality was excellent from the material we have collected and we expect to be able to analyze many more malignant peripheral nerve sheath tumors in the next year. Our experience with in situ hybridization will allow us to look at larger numbers of genes in more detail with a faster turnaround.

REFERENCES:

None.

APPENDICES

Figure 1.

Table 1.

Preprint The novel marker, DOG1, is expressed ubiquitously in GI stromal tumors irrespective of KIT or PDGFRA mutation status. *American Journal of Pathology*, in press.

Curriculum vitae Matt van de Rijn.

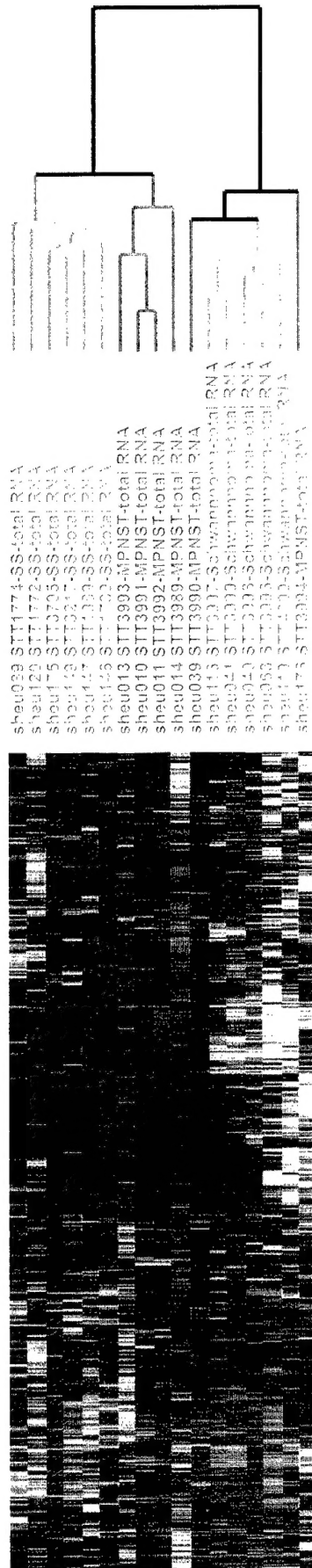


Figure 1 DAMD-17-03-1-0297

Legend to Figure 1

Unsupervised hierarchical clustering of gene expression profiling data performed on six synovial sarcomas, six malignant peripheral nerve sheath tumors and five schwannomas. All samples were analyzed on the same print run of 40,000 element cDNA gene microarrays. Data were entered in the Stanford microarray database and gene filtering was performed as described in the text. Subsequently the data were grouped together using the Cluster program developed by Mike Eisen that tumors with similar gene expression profiles across the genes selected were grouped together and genes that showed similar expression profiles across the 17 specimens tested were grouped together. The results were depicted in a "heat map" where the intersection between a gene (in rows) and a tumor specimen (in columns) was labeled red when the gene was relatively highly expressed in that sample compared to the other samples. The intersection was labeled green when the expression was relatively low. Using this unsupervised method of analysis it is clear that all synovial sarcomas (in blue) clustered together on one branch while all schwannomas (in green) likewise cluster on a separate branch. The majority of the MPNSTs tested so far (four cases) cluster on a branch separate from all other cases while the remaining two MPNST cases cluster on separate branches of the schwannoma group. By inspecting the heat map it can be seen that, as expected, the malignant peripheral nerve sheath tumors and the synovial sarcomas share expression in a large number of genes. However there is a significant number of genes that are unique to the MPNSTs and it will be these genes that will be initially studied in more detail if they can be verified in gene microarray experiments larger numbers of MPNSTs. The SAM analysis (Table 1) highlights 112 genes that are significant in distinguishing the 6 MPNSTs from the other specimens.

TABLE 1

Significant Genes List

Input Parameters

Imputation Engine
Data Type
Data in log scale?
Number of Permutations
Blocked Permutation?
RNG Seed
(Delta, Fold Change)
(Upper Cutoff, Lower Cutoff)

10-Nearest Neighbor Imputer
Two Class, unpaired data
TRUE
1000
FALSE
1234567
(0.40798,)
(1.09743, -0.4)

Computed Quantities

Computed Exchangeability Factor S0
S0 percentile
False Significant Number (Median, 90 percentile)
False Discovery Rate (Median, 90 percentile)
P0Hat

0.945709644
0.82
(7.08750, 29.92500)
(6.32812, 26.71875)
0.7875

112 Positive Significant Genes

Row	Gene Name
439	112983 POSTN perostin, osteoblast specific factor Hs.136348
441	112249 LRRRC17 leucine rich repeat containing 17 Hs.288720
441	220604 CTRHC1 collagen triple helix repeat containing 1 Hs.283713
440	308892 POSTN perostin, osteoblast specific factor Hs.136348
413	310753 LRRRC17 leucine rich repeat containing 17 Hs.288720
369	99553 RGS4 regulator of G-protein signalling 4 Hs.386726
196	104516 PTN pleiotrophin (heparin binding growth factor 8, neurite growth-promoting factor 1) Hs.44
352	99425 Transcribed sequences Hs.525878
595	114642 EGFR epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian) Hs.77432
1326	117895 HFL1 H factor (complement)-like 1 Hs.256941
194	106895 C17 cytokine-like protein C17 Hs.13872
211	119739 KIAA2028 similar to PH (pleckstrin homology) domain Hs.255938
412	110424 XRCC1 X-ray repair complementing defective repair in Chinese hamster cells 1 Hs.98483
402	248866
1329	107265 COL12A1 collagen, type XII, alpha 1 Hs.101302
401	224472 Transcribed sequence with strong similarity to protein pdb:1BGM (E. coli) O Chain O, Beta-Galactosidase Hs.387246
206	119876 MRNA full length insert cDNA clone EUROMAGE 994183 Hs.71947
347	222205 SEMA3A sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A Hs.252451
1735	115674 TM4SF9 transmembrane 4 superfamily member 9 Hs.8037
1742	99502
1744	106847 NRN1 neuritin 1 Hs.103291
403	101078
1330	111655 TIEG **TGFβ inducible early growth response Hs.82173
596	309899 EGFR epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian) Hs.77432
594	108730 CDNA: FLJ23165 fis, clone LNC09846 Hs.279898
397	104835 MYO1B myosin IB Hs.121576
1307	111660 MRNA full length insert cDNA clone EUROMAGE 248114 Hs.231971
678	120018 C5orf13 chromosome 5 open reading frame 13 Hs.508741
726	108290 NOPE likely ortholog of mouse neighbor of Punc E11 Hs.20924
351	222443 KIAA0922 KIAA0922 protein Hs.511944
438	107998 FAP fibroblast activation protein, alpha Hs.438852
407	311649 ASAM adipocyte-specific adhesion molecule Hs.135121
597	99435 EGFR epidermal growth factor receptor (erythroblastic leukemia viral (v-erb-b) oncogene homolog, avian) Hs.77432
699	109176

Gene ID	Score(d)	Numerator(t)	Denominator(s+0)	Fold Change	q-value (%)
1	2.49895525	4.562909091	1.825919183	9.47673	1.96875
1	2.237558482	3.650363636	1.631331888	16.83591	1.96875
1	2.144621646	3.422257576	1.595739548	8.73993	1.96875
1	2.131218847	3.550030303	1.689950552	8.70109	1.96875
1	2.127896524	3.447015152	1.619916717	13.42531	1.96875
1	2.07153401	3.819727273	1.843912412	8.10503	1.96875
1	2.03030837	3.179242424	1.565891404	7.77272	1.96875
1	1.90683192	3.398545455	1.782251513	8.73941	1.96875
1	1.811998976	3.373045455	1.861505166	8.09617	1.96875
1	1.801222467	3.428333333	1.903336981	5.55673	1.96875
1	1.793134532	2.800863636	1.561993027	7.64537	1.96875
1	1.772994628	3.801909091	2.144343266	6.26947	1.96875
1	1.730029111	2.643560606	1.528044002	8.34350	1.96875
1	1.723287628	2.612772727	1.516155913	4.88238	1.96875
1	1.719152154	2.741515152	1.5946950234	6.10305	1.96875
1	1.678334392	3.064227273	1.825754952	4.06973	1.96875
1	1.651920855	2.532333333	1.532962869	5.40119	1.96875
1	1.645727371	2.917272727	1.772634264	5.03821	1.96875
1	1.636089944	2.910636364	1.779019775	4.53157	1.96875
1	1.610966209	2.395787879	1.487174508	4.26634	1.96875
1	1.604237802	3.225530303	2.010631029	2.82984	1.96875
1	1.584066494	2.406257576	1.519038237	4.05597	1.96875
1	1.58137057	2.28969697	1.4479193	4.43302	1.96875
1	1.54841888	2.905151515	1.876205175	8.47433	1.96875
1	1.537913074	3.195030303	2.077510334	12.71387	1.96875
1	1.53624003	2.426090909	1.579239482	5.23870	1.96875
1	1.53092024	2.850681818	1.731430383	4.72455	1.96875
1	1.527209387	2.797121212	1.831524371	3.39428	1.96875
1	1.526266455	2.944848485	1.928445855	5.26460	1.96875
1	1.525653482	2.254878788	1.477975709	4.58324	1.96875
1	1.486713433	2.510151515	1.688389611	4.01014	1.96875
1	1.477668684	3.034666667	2.05368543	4.79512	1.96875
1	1.473733265	2.872454545	1.949100705	7.01630	1.96875
1	1.440807558	3.242121212	2.250211136	2.50095	1.96875

1737	117428	TM4SF9 transmembrane 4 superfamily member 9 Hs. 8037	1	1.420551688	2.709212121	1.907154906	8.31670	1.96875
247	106800	SYTL4 synaptolagmin-like 4 (granuphilin-a) Hs. 376981	1	1.39404643	2.39369697	1.716100725	3.14201	1.96875
1325	118248	HFL1 H factor (complement)-like 1 Hs. 298941	1	1.385715426	2.530166667	1.825991968	3.42071	1.96875
357	311130	HAS2 hyaluronan synthase 2 Hs. 159226	1	1.381114398	2.463939394	1.784022658	11.94781	1.96875
731	221983	KLHL8 kelch-like 8 (Drosophila) Hs. 372101	1	1.3681675	1.904515152	1.392018997	3.85484	1.96875
1290	113985	Clone IMAGE:5278074, mRNA Hs. 26409	1	1.368200107	2.675772727	1.958551104	2.09850	1.96875
1327	307159	HFI1 H factor 1 (complement) Hs. 278583	1	1.351882387	2.524242424	1.867205645	3.45008	2.91666667
345	105845	COL1A2 collagen, type I, alpha 2 Hs. 232115	1	1.344539149	2.079151515	1.546367405	4.21749	2.91666667
610	107468	STEAP1 six transmembrane epithelial antigen of the prostate Hs. 61635	1	1.3411171642	2.561909091	1.910202252	3.48179	2.91666667
408	116073	ASAM adipocyte-specific adhesion molecule Hs. 135121	1	1.334969315	2.287909091	1.713828973	4.01309	2.91666667
250	108540	MLSTD1 male sterility domain containing 1 Hs. 134497	1	1.333711478	2.02119697	1.515467928	4.47635	2.91666667
579	116829	IGF2 insulin-like growth factor 2 (somatomedin A) Hs. 349109	1	1.316475972	2.326136364	1.768941755	3.53605	2.91666667
139	102761	EDG2 endothelial protein DKFZp686A17109 Hs. 369523	1	1.315124404	2.010787879	1.528971611	4.48920	2.91666667
1280	118070	DKF2 dickkopf homolog 2 (Xenopus laevis) Hs. 211869	1	1.310611227	2.953333333	2.253401521	2.23197	2.91666667
445	103381	PLOD2 procollagen-lysine, 2-oxoglutarate 5-dioxygenase (lysine hydroxylase) 2 Hs. 41270	1	1.294390952	1.775424242	1.371629058	3.69645	2.91666667
417	223884	PRRX1 paired related homeobox 1 Hs. 443452	1	1.292280155	2.605757576	2.016402996	3.32533	2.91666667
1738	226503	C1orf24 chromosome 1 open reading frame 24 Hs. 48778	1	1.291536368	2.404848485	1.862006014	3.21995	2.91666667
199	101968	SPRY2 sprouty homolog 2 (Drosophila) Hs. 18676	1	1.289037991	2.20030303	1.706934198	4.46361	2.91666667
443	116402	MPRA: cDNA DKFZp686G03142, (from clone DKFZp686G03142) Hs. 289044	1	1.275687569	1.74030303	1.364314824	3.41619	4.00423729
361	100151	LFR leukemia inhibitory factor receptor Hs. 446501	1	1.274858688	1.899560606	1.490018726	3.63359	4.00423729
187	110597	NEF3 neurofilament 3 (150kDa medium) Hs. 458657	1	1.265827763	2.375030303	1.876265651	4.48930	4.00423729
727	115261	ENAH enabled homolog (Drosophila) Hs. 446693	1	1.254547395	2.363212121	1.883716893	3.09159	4.00423729
373	317940	ITGA4 integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor) Hs. 528404	1	1.253590071	2.298121212	1.833318623	3.14574	4.00423729
653	319689	CDNA clone MGC-52283 IMAGE:4123447, complete cds Hs. 251684	1	1.248032848	2.540909091	2.035931262	2.99297	4.25675676
91	118015	PEG10 paternally expressed 10 Hs. 137476	1	1.244945006	2.407530303	1.9338447	3.28927	4.25675676
410	111639		1	1.243928797	1.876060606	1.508173628	4.07571	4.25675676
424	119328	RAB23 RAB23, member RAS oncogene family Hs. 94769	1	1.236085487	1.771484848	1.433141046	3.25811	4.25675676
212	100732	ECM2 extracellular matrix protein 2, female organ and adipocyte specific Hs. 117060	1	1.236081775	2.134242424	1.726619119	4.03006	4.25675676
598	101379		1	1.233924901	2.226121212	1.804097811	3.79646	4.25675676
197	100071	Transcribed sequences Hs. 32135	1	1.233257794	2.349909091	1.9054484	3.67514	4.25675676
634	101410	IQGAP3 IQ motif containing GTPase activating protein 3 Hs. 133294	1	1.229481227	2.220833333	1.806317401	3.48407	4.25675676
367	311177	LOX lysyl oxidase Hs. 102267	1	1.225014059	1.744090909	1.423731341	2.98819	4.25675676
205	104532	STXBP6 syntaxin binding protein 6 (amisyn) Hs. 99291	1	1.222217886	1.826272727	1.494228442	3.30081	4.25675676
435	106250	TMEM2 transmembrane protein 2 Hs. 160417	1	1.217934553	2.109393939	1.731943587	3.40537	4.25675676
1328	119752	DKFZP586L151 DKFZP586L151 protein Hs. 43658	1	1.213684925	2.070878788	1.706273798	3.18066	4.25675676
1023	110878	DOCK11 dedicator of cytokinesis 11 Hs. 107513	1	1.21129016	2.322424242	1.900310207	2.50462	4.25675676
515	105460	WDTG1 **WD and tetrahydropeptide repeats 1 Hs. 172825	1	1.207685873	1.819696997	1.506763481	2.85151	4.25675676
404	330989	Similar to pyruvate-5-carboxylate reductase 1 isoform 2; P5C reductase (LOC400634), mRNA Hs. 512314	1	1.207015281	1.766212121	1.463288948	2.66131	4.25675676
454	105760	DDEF1 development and differentiation enhancing factor 1 Hs. 398779	1	1.202432032	1.869060606	1.554400212	3.43121	5.22321429
358	115196	LOX lysyl oxidase Hs. 102267	1	1.19572801	2.137878788	1.787930675	4.30948	5.22321429
437	107150	CDNA FLJ35517 fs, clone SPLEN2000698 Hs. 519270	1	1.187400027	1.746196997	1.470605485	2.88180	5.22321429
427	108628	ADAM12 a disintegrin and metalloproteinase domain 12 (meltrin alpha) Hs. 8850	1	1.185192406	1.731515152	1.460957009	3.33179	5.22321429
516	106445	MGC9850 hypothetical protein MGC9850 Hs. 222061	1	1.178616151	1.968181818	1.689909085	2.94614	5.22321429
677	111520	MRNA: cDNA DKFZp586L0120 (from clone DKFZp586L0120) Hs. 98314	1	1.173921935	2.153939394	1.834823364	2.79660	5.22321429
1256	100028	CLEC3F2 **C-type (calcium dependent, carbohydrate-recognition domain) lectin, superfamily member 2 (activation-induced) Hs. 85201	1	1.173326924	2.774454545	2.000682417	2.36489	5.22321429
616	99493	CDCA1 cell division cycle associated 1 Hs. 234545	1	1.172412625	1.747424242	1.516039835	2.41316	5.22321429
409	311729	CXCL6 chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2) Hs. 164021	1	1.166683601	1.825181818	1.564418851	2.99191	5.22321429
651	116998	CDCR coiled-coil domain containing 8 Hs. 97876	1	1.160548388	1.889924242	1.628475178	3.30904	5.22321429
425	101543	ADAM12 a disintegrin and metalloproteinase domain 12 (meltrin alpha) Hs. 8850	1	1.157030094	1.605909091	1.387238548	3.15881	5.22321429
360	106544	SMT2 **serine hydroxymethyltransferase 2 (mitochondrial) Hs. 75069	1	1.152617738	1.715106061	1.48373636	3.24975	5.22321429
257	104207	FZD6 frizzled homolog 6 (Drosophila) Hs. 114218	1	1.155616781	2.379242424	2.058847142	1.82285	5.22321429
350	103810	PDE9B phosphodiesterase 8B Hs. 78106	1	1.154976376	1.870696997	1.619684184	2.95897	5.22321429
213	98915	OGN osteoglycin (osteoinductive factor, mimecan) Hs. 109439	1	1.154810442	1.996212121	1.728609881	3.17579	5.22321429
1736	312147	TM4SF9 transmembrane 4 superfamily member 9 Hs. 8037	1	1.151726636	1.963166667	1.704542211	3.02629	5.22321429
			1	1.150375978	2.302545455	2.001559054	6.90794	5.22321429
			1	1.149928511	2.100151515	1.8263332242	4.57315	5.22321429

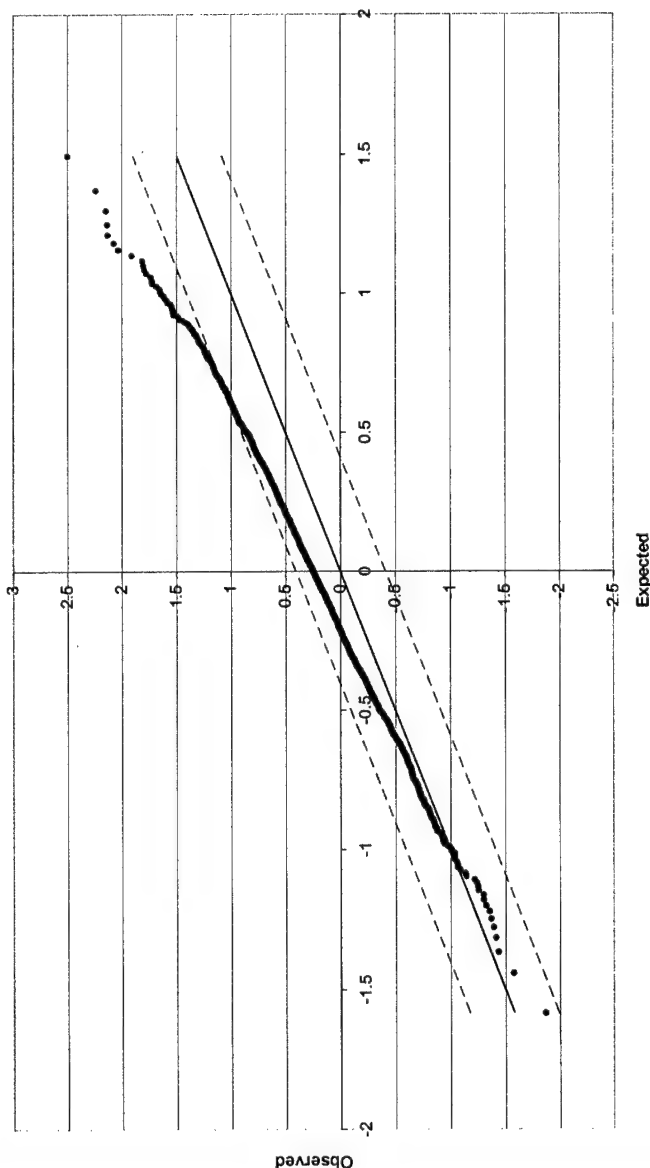
Table 1 - page three of three

387 100457 MBNL1 muscleblind-like (Drosophila) Hs.28578	1	1.147032892	1.945757576	1.696339825	9.26944	5.22321429
1331 309530 CXCL14 chemokine (C-X-C motif) ligand 14 Hs.24395	1	1.146247354	2.257090909	1.969113299	3.94158	5.22321429
202 310309 ENPP2 ectonucleotide pyrophosphatase/phosphodiesterase 2 (autotaxin) Hs.23719	1	1.145241645	1.933545455	1.688329675	4.20937	5.22321429
782 114093 FZD3 frizzled homolog 3 (Drosophila) Hs.40735	1	1.141748823	1.784833333	1.563245171	2.00932	5.22321429
1146 319580 FST follistatin Hs.5914	1	1.141640136	1.895333333	1.561060498	4.73318	5.22321429
447 187712 STK38L serine/threonine kinase 38 like Hs.184523	1	1.141562622	1.868727273	1.538905953	4.88712	5.22321429
392 117540 Transcribed sequences Hs.128809	1	1.136857639	1.754409091	1.543209133	2.79309	5.30048077
998 109251 PRKQ protein kinase C, theta Hs.408049	1	1.131628031	2.198772727	1.943017199	3.18905	5.30048077
249 330551 PRKQ protein kinase C, theta Hs.408049	1	1.12688191	2.063	1.931202846	17.03237	5.30048077
370 100008 GSF10 immunoglobulin superfamily, member 10 Hs.386296	1	1.125401978	1.818287879	1.514244217	3.15050	5.30048077
687 111691 EDNRA endothelin receptor type A Hs.211202	1	1.125991005	1.913545455	1.69432275	4.28225	5.30048077
1324 109102 MCP matrix Gla protein Hs.365706	1	1.122712847	2.11830303	1.886771881	2.37488	5.30048077
1026 103897 RHOBTB3 Rho-related BTB domain containing 3 Hs.31653	1	1.113778714	1.734212121	1.55705267	3.26273	6.328125
385 330992 C3BP2 Ras-GTPase activating protein SH3 domain-binding protein 2 Hs.303878	1	1.106040355	1.625575758	1.469725539	3.84376	6.328125
512 307378 STC1 stanniocalcin 1 Hs.25590	1	1.104105893	1.783257576	1.615114626	3.87858	6.328125
499 314788 CDNA FLJ11501 fis, clone BRTHA2006975 Hs.389638	1	1.103899985	1.726939394	1.564398422	2.22770	6.328125
446 99834	1	1.099215031	1.570787879	1.429008732	3.39925	6.328125
593 100664 SVIL supervillin Hs.163111	1	1.098954578	1.916121212	1.743585449	2.16294	6.328125
428 226520 ADAM12 a disintegrin and metalloproteinase domain 12 (meltrin alpha) Hs.8850	1	1.098456828	1.794969697	1.634083062	3.07196	6.328125
434 102456 PTGIS prostaglandin I2 (prostaglandin) synthase Hs.302085	1	1.09742759	1.695015152	1.544534844	2.44386	6.328125

Significant: 112
Median # false significant: 7.08750

Delta 0.40799
Fold Change

SAM Plot



The novel marker, *DOG1*, is expressed ubiquitously in GI Stromal Tumors
irrespective of *KIT* or *PDGFRA* mutation status

Robert B. West¹, Christopher L. Corless², Xin Chen⁷, Brian P. Rubin³, Subbaya
Subramanian¹, Kelli Montgomery¹, Shirley Zhu¹, Catherine A. Ball⁴, Torsten O.
Nielsen⁵, Rajiv Patel⁶, John R. Goldblum⁶, Patrick O. Brown⁷, Michael C. Heinrich⁸,
Matt van de Rijn¹.

Departments of ¹Pathology, ⁴Genetics, and ⁷Biochemistry and Howard Hughes
Medical Institute, Stanford University Medical Center, Departments of ²Pathology
and ⁸Medicine, Oregon Health & Science University Cancer Institute and Portland
VA Medical Center, Portland, OR, ³Department of Anatomical Pathology,
University of Washington Medical Center, Seattle, WA, ⁵Department of Pathology
and Genetic Pathology Evaluation Centre, Vancouver General Hospital, Vancouver
BC, ⁶Dept of Anatomic Pathology, Cleveland Clinic Foundation, Cleveland, OH.

Address correspondence to:

Matt van de Rijn: mrrijn@stanford.edu

Department of Pathology, Stanford University Medical Center

300 Pasteur Drive, Stanford, CA, 94305

Phone: 650-498-7154

Abstract:

We recently characterized gene expression patterns in GISTs using cDNA microarrays, and found that the gene *FLJ10261* (DOG1, Discovered on GIST-1), encoding a hypothetical protein, was specifically expressed in GISTs. The immunoreactivity of a rabbit antiserum to synthetic DOG1 peptides was assessed on two soft tissue tumor microarrays (TMAs). The TMAs included 587 soft tissue tumors, with 149 GISTs, including 127 GIST cases for which the *KIT* and *PDGFRA* mutation status was known. Immunoreactivity for DOG1 was found in 136 of 139 (97.8%) of scorable GISTs. All 7 GIST cases with a *PDGFRA* mutation were DOG1 positive, while most of these failed to react for KIT. The immunohistochemical findings were confirmed with in situ hybridization probes for *DOG1*, *KIT* and *PDGFRA*. Other neoplasms in the differential diagnosis of GIST, including desmoid fibromatosis (0/17) and Schwannoma (0/3), were immunonegative for DOG1. Only 4 out of 438 non-GIST cases were immunoreactive for DOG1.

DOG1, a protein of unknown function, is expressed strongly on the cell surface of GISTs and is rarely expressed in other soft tissue tumors. Reactivity for DOG1 may aid in the diagnosis of GISTs, including *PDGFRA* mutants that fail to express KIT antigen, and lead to appropriate treatment with imatinib mesylate, an inhibitor of the KIT tyrosine kinase.

Introduction:

Gastrointestinal stromal tumors occur in the wall of the bowel and have been proposed to arise from the interstitial cells of Cajal. The differential diagnosis of these tumors includes desmoid fibromatosis, Schwannoma, leiomyosarcoma, and, in some cases, high grade sarcomas ¹. Accurate diagnosis of GIST is important, because imatinib mesylate has been shown to significantly inhibit these tumors presumably through inhibition of the KIT tyrosine kinase receptor, which is highly expressed in these tumors ²⁻⁵. As a result, the diagnosis of GIST relies heavily on KIT immunoreactivity. Current recommendations in the literature emphasize a diffuse, strong KIT immunoreactivity for the diagnosis of GIST ⁶. CD34 immunostaining can also aid in the diagnosis, but a subset of cases is immunonegative while many other types of sarcomas are immunoreactive for this marker ⁷⁻¹⁰. In the vast majority of GISTs, high levels of KIT expression are accompanied by a *KIT* gene mutation in exon 9, 11, 13 or 17 ^{11,12}.

Recently, a subset of GISTs have been found to have *PDGFRA* mutations rather than *KIT* mutations ^{13,14}. Patients with GISTs containing mutations in *PDGFRA* may still benefit from imatinib therapy, but these tumors often fail to react with antibodies against KIT and hence may remain undiagnosed as GIST ². In addition, some GISTs with *KIT* mutations may have low KIT expression by immunohistochemistry yet will still respond to imatinib therapy ¹⁵.

Although much work has been done on the biology of GISTs and KIT, additional insight has recently been gained through gene microarray studies ¹⁶⁻¹⁸. These studies have identified a number of genes whose expression is relatively increased compared to other soft tissue tumors. This includes genes known to be involved with GISTs, such as

KIT and *CD34*, but also includes a number of genes that have not been well characterized. We have generated an antiserum against one GIST specific gene, encoding for the hypothetical protein FLJ10261, which we have named "Discovered on GIST 1" (DOG1). Using immunohistochemistry with this antiserum and in situ hybridization with *DOG1*-specific probes, we show that DOG1 is highly expressed not only in typical GISTs but also in *KIT*-mutation negative GIST.

Materials and Methods:

Tissue Microarray

The studies described here were performed with the approval of the Institutional Review Board at Stanford University Hospital. Two TMAs were used for this study. The first TMA contained 460 different soft tissue tumors from 421 patients, with each tumor represented by two cores. The samples were distributed over two array blocks that were constructed using a technique previously described¹⁹ with a tissue arrayer from Beecher Instruments, Silver Spring, MD. 0.6 mm cores were taken from paraffin embedded soft tissue tumors archived from the Stanford University Medical Center between 1995 and 2001. This array has also been used for characterization of Apolipoprotein D expression²⁰. The second TMA used GISTs that were obtained from the pathology archives of Oregon Health and Science University Hospital, the Portland VA Medical Center and the Kaiser Permanente Northwest Regional Laboratory. This single-block array consisted of 0.6 mm cores from formalin-fixed, paraffin-embedded tumor assembled using a semi-automated tissue arrayer²¹. There was one core for each tumor, and all of the GISTs on this TMA were analyzed for mutations in exons 9, 11, 13 and 17 of the *KIT* gene using a combination of denaturing HPLC and direct sequencing, as previously described^{13,22}. *KIT* wild-type tumors included on the array were also screened for mutations in exons 12 and 18 of the *PDGFRA* gene¹³.

Antibody Generation

The cDNA-derived protein sequence of *DOG1* showed no significant homology with other genes, including the *KIT* gene. A rabbit polyclonal antibody was raised by

injecting 3 peptides derived from the gene sequence (Applied Genomics Inc. (AGI), Huntsville, AL). These peptides have no sequence homology to KIT. The peptides were synthesized by standard Fmoc chemistry: Peptide 1 EEAVKDHPRAEYEARVLEKSLK; Peptide 2 DHEECVKRKQRYEVDYNLE; Peptide 3 KEKVLMVELFMREEQDK. The peptides were conjugated to KLH and injected into two out-bred rabbits. The serum (S284) was harvested after the rabbits demonstrated a significant anti-peptide titer. Affinity-purified antibodies were obtained by passing the antiserum over an affinity column conjugated with the three peptides; bound antibodies were eluted with a pH gradient.

Immunohistochemistry

Primary antibodies were directed towards DOG1 (S284, AGI, Rabbit polyclonal, 1:50) and KIT (DAKO, Carpinteria, CA Rabbit polyclonal, 1:50). Serial sections of 4 μ M were cut from the tissue array blocks, deparaffinized in xylene, and hydrated in a graded series of alcohol. Staining was then performed using the EnVision+ anti-rabbit system (DAKO).

In situ hybridization

In situ hybridization of TMA sections was performed based on a protocol published previously^{23,24}. Briefly, digoxigenin (DIG)-labeled sense and anti-sense RNA probes are generated by PCR amplification of 400 to 600 bp products with the T7 promoter incorporated into the primers. In vitro transcription was performed with a DIG RNA-labeling kit and T7 polymerase according to the manufacturer's protocol (Roche

Diagnostics, Indianapolis, IN). 5µm thick sections cut from the paraffin blocks, deparaffinized in xylene, were hydrated in graded concentrations of ethanol for 5 minutes each. Sections were then incubated with 1% hydrogen peroxide, followed by digestion in 10µg/ml of proteinase K at 37°C for 30 minutes. Sections were hybridized overnight at 55°C with either sense or antisense riboprobes at 200ng/ml dilution in mRNA hybridization buffer (Dako). The following day, sections were washed in 2xSSC and incubated with 1:35 dilution of RNase A cocktail (Ambion, Austin, TX) in 2xSSC for 30 minutes at 37°C. Next, sections were stringently washed in 2X SSC/50% formamide twice, followed by one wash at 0.08X SSC at 50 °C. Biotin blocking reagents (Dako) were applied to the section to block the endogenous biotin. For signal amplification, a HRP-conjugated rabbit anti-DIG antibody (Dako) was used to catalyze the deposition of biotinyl tyramide, followed by secondary streptavidin complex (GenPoint kit; Dako). The final signal was developed with DAB (GenPoint kit; Dako), and the tissues were counterstained in hematoxylin for 15 seconds.

Scoring of Immunohistochemistry and in situ hybridization

Cores were scored as follows. A score of "0" was given for absent or insignificant staining: less than 5% tumor cells with light brown staining. A score of "1" was given for unscorable cores. A score of "2" was given for light brown stain in greater than 5% of tumor cells or dark brown stain in less than 50% of tumor cells. A score of "3" was given for dark brown staining in greater than 50% tumor cells. Non-tumor cells and cells of unknown origin were not scored. The cores were independently reviewed by two

pathologists (RBW and MvdR) and disagreements were reviewed together to achieve a consensus score.

Digital image collection and data analysis

To aid in the analysis of numerous tissue cores stained by immunohistochemistry and in situ hybridization, digital images were collected using the BLISS instrument (Bacuslabs, Lombard IL; <http://bacuslabs.com>). Scoring results were combined using Deconvoluter and represented in Treeview²⁵, as shown on the accompanying website (http://microarray-pubs.stanford.edu/tma_portal/dog1/), where over 4,000 digital images are available.

Results:

Previously, we examined the gene expression profile of GISTs using cDNA microarrays and identified a number of the genes, in addition to the *KIT* gene, that demonstrated a specific pattern of elevated mRNA expression in GISTs¹⁸. Figure 1 shows the relative level of mRNA expression for one of these genes, *DOG1 (FLJ10261)*, compared with *KIT* in a variety of soft tissue tumors, including those in the differential diagnosis of GIST. Searches failed to show any sequence similarity between the genes on either the DNA or protein level.

A rabbit antiserum was generated against synthetic peptides derived from the putative coding sequence of DOG1. Antiserum immunoreactivity was characterized on two separate TMAs containing soft tissue tumors. The first TMA contained 460 different soft tissue tumor samples representing over 50 different diagnostic entities²⁰. This array included 22 KIT-immunoreactive GISTs. The second TMA included 127 GIST cases for which the *KIT* and *PDGFRA* mutation status was previously determined. On this TMA there were 102 cases with an activating mutation in *KIT*, 8 cases with a mutation in *PDGFRA*, and 17 cases that were wild-type for both kinases but nevertheless had clinical, histologic, and immunophenotypic features typical for GIST.

In these two TMAs, 136 of 139 scorable GISTs (97.8%) demonstrated immunoreactivity with DOG1 antiserum (Figures 2 and 3, Table 1). The staining observed with DOG1 antisera appeared predominately localized to the plasma membrane (Figure 4A). In some very strongly immunoreactive samples, the subcellular distribution of the staining could not be evaluated (Figure 4B). Mast cells present in some of the samples, for example synovial sarcoma, were strongly immunoreactive as well (Figure

4C), while the same samples showed only weak staining in the mast cells with KIT antibodies. We confirmed these results with in situ hybridization studies (Figures 5 and 6). Interestingly, DOG1 antisera stained all 8 scorable *PDGFRA*-mutant GISTs (1 case from 1st TMA and 7 cases from 2nd TMA), while the KIT antibody staining was weak in 3 of these cases and negative in the remaining 5. These findings were further extended by in situ hybridization with *PDGFRA* (Figure 6). *PDGFRA* expression was predominately, but not exclusively, present in the *PDGFRA*-mutant GISTs. 5 of 6 (83%) scorable *PDGFRA*-mutant GISTs were positive for *PDGFRA* ISH (Figure 2 and 3, table 1). In contrast, only 10 of 70 (14%) *KIT*-mutant and *KIT*-wildtype GISTs were positive for *PDGFRA* ISH. Correlation of *KIT* ISH with KIT immunohistochemistry was good, with the ISH signal detectable in almost all immunopositive cases (Figure 2). However, a difference was seen in the *PDGFRA*-mutant GISTs with regard to KIT expression. Three cases were immunopositive for KIT, but only one case was positive by *KIT* ISH. Hierarchical clustering analysis of IHC and ISH data was performed as previously described²⁵. Among these parameters – KIT IHC, *KIT* ISH, DOG1 IHC, *DOG1* ISH, and *PDGFRA* ISH – the most distinguishing feature was *PDGFRA* ISH positivity (Figure 2), with overexpression of PDGFRA by *PDGFRA* ISH seen in only in a small subset of GISTs. Images of all cores from both TMAs were digitally captured and are available at the accompanying website (http://microarray-pubs.stanford.edu/tma_portal/dog1/).

From the 460 tumor samples that were not classified as GIST in the first TMA, only four cases that were not histologically and immunophenotypically consistent with GIST were immunoreactive with DOG1 antiserum: 1 synovial sarcoma (1/20 = 5%), 1 (1/40 = 2.5%) leiomyosarcoma, 1 (1/4 = 25%) fibrosarcoma, and (1/9 = 11%) 1 Ewing's

sarcoma/PNET. Of the 40 leiomyosarcomas, 17 originated in the abdomen and none of these were DOG1 immunoreactive. Other tumors in the GIST differential diagnosis failed to stain with the DOG1 antisera. These include desmoid fibromatosis (17 cases) and Schwannoma (3 cases). Parenthetically, under the staining conditions used, none of the fibromatosis cases were positive for KIT by immunohistochemistry or in situ hybridization. One leiomyosarcoma was positive for KIT immunohistochemistry only (TMA 3725). Interestingly, the staining was exclusively in a diffuse nuclear pattern. This tumor was negative for DOG1 by both immunohistochemistry and in situ hybridization and for *KIT* in situ hybridization.

Seven cases in the first TMA, not counted among the 22 unequivocal GISTs, showed histologic features indeterminate between GIST and smooth muscle tumor. All of these tumors were located in the wall of the stomach or intestine, with four tumors from the stomach, one from the duodenum, one from the gastro-esophageal junction, and one from the rectum. All seven cases were negative for KIT by immunohistochemistry and thus might not be considered GISTs according to current recommendations⁶. However, four of the seven cases were positive by *KIT* in situ hybridization, while DOG1 immunoreactivity was seen in two cases, and all seven cases were positive for *DOG1* by in situ hybridization. Furthermore, two cases (TMA 863 and 3696) were positive for *PDGFRA* in situ hybridization. Subsequent sequence analysis of cases 863 and 3696 revealed a point mutation and a deletion in exon 18 of *PDGFRA*, respectively. To date, such mutations have only been described in GISTs. We conclude that the seven KIT immunonegative cases with morphologic features between GIST and smooth muscle tumor actually represent GISTs.

We also stained a tissue microarray containing a spectrum of normal tissues with the DOG1 antiserum (data not shown). We observed staining in the epithelium of breast, prostate, salivary gland, liver, stomach, testis, pancreas, and gallbladder. The pattern of DOG1 immunostaining of the Interstitial Cells of Cajal was similar to KIT. In addition, DOG1 antiserum reacted with a number of tumor cores in a carcinoma array, including some that did not stain with KIT antiserum (data not shown).

Discussion:

GISTs have a high rate of local recurrence ¹. Imatinib, a small molecule inhibitor of several type III receptor tyrosine kinases, including KIT and PDGFRA, has demonstrated promise in controlling GIST growth ³⁻⁵. The majority of GISTs (80-85%) harbor oncogenic mutations of KIT, and for this reason KIT has been regarded as the primary target for imatinib therapy. Indeed, initial trials of imatinib were limited to KIT-immunoreactive GISTs. Recently it was discovered that a subset of GISTs (5-7%) has activating mutations of PDGFRA ^{13,14}. Most of these tumors are weak or negative in immunostaining for KIT, which may lead to underdiagnosis and possible withholding of imatinib therapy. Furthermore, identification of *PDGFRA*-mutant GISTs requires molecular analysis, a laborious process that is not ideal for application in a routine clinical setting.

In this paper, we demonstrate that a novel gene, *DOG1*, identified in a DNA microarray analysis of gene expression patterns as associated with GIST, is highly expressed in both *KIT*- and *PDGFRA*- mutant GISTs. Expression of DOG1 in GISTs was demonstrated both by immunodetection of the protein and by in situ hybridization. DOG1 immunoreactivity was assessed on two soft tissue tumor microarrays representing 587 soft tissue tumors, including 149 GISTs. 98.7% of scorable GISTs demonstrated immunoreactivity with DOG1 antisera. Only four KIT-negative, non-GIST soft tissue tumors were DOG1 immunoreactive. Several GISTs with mutations in the *PDGFRA* gene were found to react only by in situ hybridization for DOG1 and to be negative for DOG1 by immunohistochemistry. Future studies are necessary to determine whether monoclonal antibodies against purified DOG1 might yield tools with sensitivity similar to

that seen with in situ hybridization probes. We also confirm PDGFRA expression in a subset of GISTs using in situ hybridization. PDGRFA expression and KIT expression are not mutually exclusive. A subset of KIT-mutated GISTs expresses PDGRFA in addition to KIT while a subset of PDGRFA-mutated tumors also expresses KIT. These data were seen with both immunohistochemical and in situ hybridization techniques.

In addition to the marked similarity in reactivity for DOG1 protein on non-GIST sarcomas, DOG1 protein can also be seen in a subset of melanomas and germ cell tumors as has been described for KIT (West et al., in preparation). Furthermore just as seen with the KIT molecule, a variety of carcinomas also express DOG1. These tumors mostly overlap with the KIT positive tumors. While within the field of soft tissue tumors DOG1 expression appears quite specific for GIST, in a differential diagnostic setting DOG1 reactivity does not exclude carcinomas. Therefore additional markers such as keratin stains should be performed when the differential diagnosis includes carcinoma.

We also demonstrated the feasibility of assessing GIST markers by in situ hybridization on paraffin embedded tissue. Correlation between immunohistochemistry and ISH for DOG1 on GISTs was excellent. In the case of KIT, the correlation was not as strong due to relatively weak or absent ISH signals in some CD117-positive GISTs. It is likely that this reflects lower sensitivity of the *KIT* ISH assay, although cross-reactivity of the CD117 antibody to another epitope on GISTs has not been excluded. In situ hybridization for *PDGFRA* proved to be valuable in identifying KIT-negative GISTs, although DOG1 immunohistochemistry was equally sensitive for these cases. Overall, we have found that ISH techniques are complementary to IHC tests in the evaluation of GISTs.

DOG1 has been recently identified as a gene in the *CCND1-EMSI* locus on human chromosome 11q13, which is amplified in esophageal cancer, bladder tumors, and breast cancer²⁶. Human *DOG1* protein showed 89.8% total-amino-acid identity with mouse *DOG1* protein, and also 58.4%, 38.3%, and 38.6% identity with human C12orf3, C11orf25, and FLJ34272/BAC03704 proteins, respectively. Sequence analysis predicts the presence of eight transmembrane spanning segments. This correlates with our observations of the immunohistochemical localization to the cell membrane. *DOG1* may be part of an as yet unclassified ion transporter family.

Since the biologic function is unknown, it is unclear why *DOG1* is so widely expressed in GISTs. Two broad possibilities exist. It may be that the protein has a role in receptor kinase type III signal transduction pathways. On the other hand, *DOG1* may be a fortuitous marker of the GIST phenotype, with no direct connection to the KIT and PDGFRA signaling pathways. The finding that mast cells are also immunoreactive for *DOG1* tends to favor the former possibility.

In summary, we demonstrate that detection of a novel gene, *DOG1*, identifies the vast majority of both *KIT*- and *PDGFRA*- mutated GISTs. This may be of clinical value in identifying candidates for Gleevec therapy. As a cell membrane associated protein, with markedly elevated expression in GISTs, *DOG1* may also be a potential therapeutic target.

ACKNOWLEDGEMENTS:

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Institute.

Figure Legends.

Figure 1: Gene array measurement of *KIT* and *DOG1* mRNA expression in 30 soft tissue tumors. Red indicates a relatively high level of expression while green denotes a low level of expression. Gene array data for STTs 524, 629, 417, 418, 219, 111, 656, 94, 335, 794, 1148, 850, 616, 710, 523, 526, 740, 607, and 1220 have been previously reported¹⁸.

Figure 2: Hierarchical clustering of CD117 IHC, *CD117* ISH, *PDGFRA* ISH, DOG1 IHC, and *DOG1* ISH. The results for GISTs on the 2 TMAs have been combined. Antisera or hybridization probes are in columns, tumors in rows. Bright red denotes strong reactivity, while dark red and green indicate low and absent reactivity, respectively. White means missing data.

Figure 3: Staining results on GISTs for CD117 IHC, *CD117* ISH, *PDGFRA* ISH, DOG1 IHC, and *DOG1* ISH in graphic form (see also Table 1).

Figure 4: Immunohistochemical staining with anti-DOG1 serum (S284) and KIT on 2 GISTs (TMA 822 (A) and 3688 (B)) and a synovial sarcoma (TMA 856 (C)).

Figure 5: In situ hybridization of a GIST and leiomyosarcoma with antisense probes to *DOG1* and *KIT* on a GIST and a leiomyosarcoma (LMS). The corresponding negative control sense probes are included in the inset in the upper right hand corner of the GIST sample.

Figure 6: In situ hybridization of *KIT*, *DOG1*, and *PDGFRA* with GISTs.

A) GIST with mutation in *KIT* shows positive ISH for *KIT*, *DOG1* but not *PDGFRA*.

B) GIST with mutation in *PDGFRA* shows positive ISH for *DOG1* and *PDGFRA* but not for *KIT*.

C) Negative control leiomyosarcoma.

Tables

Table 1: Staining results for CD117 IHC, *CD117* ISH, *PDGFRA* ISH, DOG1 IHC, and *DOG1* ISH in tabular form (see also Figure 3).

Table 1

	<u>CD117</u>	<u>CD117 ISH</u>	<u>PDGFRA</u> <u>ISH</u>	<u>DOG1</u>	<u>DOG1 ISH</u>	
wt	14	10	9	14	3	total scorables
	14	9	1	14	3	total positive
	100	90	11	100	100	% positive
KIT ex 9	9	7	7	9	6	total scorables
	9	6	2	8	5	total positive
	100	86	29	89	83	% positive
KIT ex 11	86	57	51	81	39	total scorables
	82	47	6	81	38	total positive
	95	82	12	100	97	% positive
KIT ex 13	3	3	2	3	2	total scorables
	3	2	1	3	2	total positive
	100	67	50	100	100	% positive
KIT ex 17	1	1	1	1	0	total scorables
	1	1	0	1	0	total positive
	100	100	0	100	NA	% positive
PDGFRA	8	7	6	8	7	total scorables
	3	1	5	8	5	total positive
	37.5	14	83	100	71	% positive
unknown	23	23	21	23	23	total scorables
	22	21	8	21	22	total positive
	96	91	38	91	96	% positive

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3. van Oosterom AT, Judson I, Verweij J, Stroobants S, Donato di Paola E, Dimitrijevic S, Martens M, Webb A, Sciort R, Van Glabbeke M, Silberman S, Nielsen OS, Group EOfRaToCSTaBS: Safety and efficacy of imatinib (STI571) in metastatic gastrointestinal stromal tumours: a phase I study. *Lancet* 2001, 358:1421-1423
4. Demetri G, von Mehren M, Blanke C, Van den Abbeele A, Eisenberg B, Roberts P, Heinrich M, Tuveson D, Singer S, Janicek M, Fletcher J, Silverman S, Silberman S, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker B, Corless C, Fletcher C, Joensuu H: Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002, 347:472-480
5. Joensuu H, Roberts P, Sarlomo-Rikala M, Andersson L, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B, Demetri G: Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* 2001, 344:1052-1056
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8. van de Rijn M, Hendrickson MR, Rouse RV: CD34 expression by gastrointestinal tract stromal tumors. *Hum Pathol* 1994, 25:766-771
9. Yantiss RK, Spiro IJ, Compton CC, Rosenberg AE: Gastrointestinal stromal tumor versus intra-abdominal fibromatosis of the bowel wall: a clinically important differential diagnosis. *Am J Surg Pathol* 2000, 24:947-957
10. Smithey BE, Pappo AS, Hill DA: C-kit expression in pediatric solid tumors: a comparative immunohistochemical study. *Am J Surg Pathol* 2002, 26:486-492
11. Hirota S, Nishida T, Isozaki K, Taniguchi M, Nakamura J, Okazaki T, Kitamura Y: Gain-of-function mutation at the extracellular domain of KIT in gastrointestinal stromal tumours. *J Pathol* 2001, 193:505-510
12. Rubin B, Singer S, Tsao C, Duensing A, Lux M, Ruiz R, Hibbard M, Chen C, Xiao S, Tuveson D, Demetri G, Fletcher C, Fletcher J: KIT activation is a ubiquitous feature of gastrointestinal stromal tumors. *Cancer Res* 2001, 61:8118-8121

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14. Hirota S, Ohashi A, Nishida T, Isozaki K, Kinoshita K, Shinomura Y, Kitamura Y: Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. *Gastroenterology* 2003, 125:660-667
15. Bauer S, Corless C, Heinrich M, Dirsch O, Antoch G, Kanja J, Seeber S, Schutte J: Response to imatinib mesylate of a gastrointestinal stromal tumor with very low expression of KIT. *Cancer Chemother Pharmacol* 2003, 51:261-265
16. Allander SV, Nupponen NN, Ringner M, Hostetter G, Maher GW, Goldberger N, Chen Y, Carpten J, Elkahoul AG, Meltzer PS: Gastrointestinal stromal tumors with KIT mutations exhibit a remarkably homogeneous gene expression profile. *Cancer Res* 2001, 61:8624-8628
17. Khan J, Wei JS, Ringner M, Saal LH, Ladanyi M, Westermann F, Berthold F, Schwab M, Antonescu CR, Peterson C, Meltzer PS: Classification and diagnostic prediction of cancers using gene expression profiling and artificial neural networks. *Nat Med* 2001, 7:673-679
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20. West RB, Harvell J, Linn S, Lui C, Prapong W, Hernandez-Boussard T, Montgomery K, Nielsen TO, Rubin BP, Patel R, Goldblum JR, Brown P, van de Rijn M: Apo D in Soft Tissue Tumors: a Novel Marker for Dermatofibrosarcoma Protuberans. *Am J Surg Pathol* In press
21. Torhorst J, Bucher C, Kononen J, Haas P, Zuber M, Kochli O, Mross F, Dieterich H, Moch H, Mihatsch M, Kallioniemi O, Sauter G: Tissue microarrays for rapid linking of molecular changes to clinical endpoints. *Am J Pathol* 2001, 159:2249-2256
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24. Iacobuzio-Donahue CA, Ryu B, Hruban RH, Kern SE: Exploring the host desmoplastic response to pancreatic carcinoma: gene expression of stromal and neoplastic cells at the site of primary invasion. *Am J Pathol* 2002, 160:91-99
25. Liu CL, Prapong W, Natkunam Y, Alizadeh A, Montgomery K, Gilks CB, van de Rijn M: Software tools for high-throughput analysis and archiving of immunohistochemistry staining data obtained with tissue microarrays. *Am J Pathol* 2002, 161:1557-1565

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Figure 1

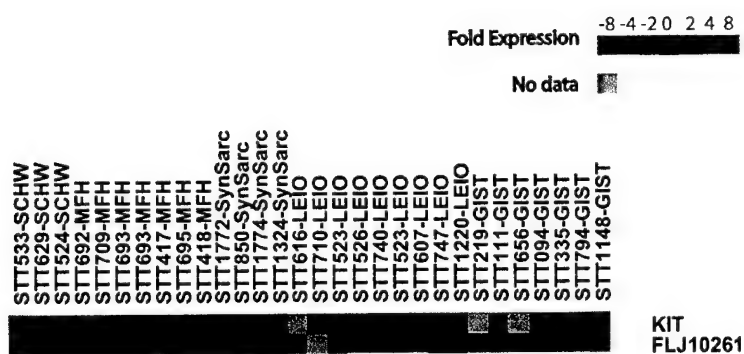


Figure 2

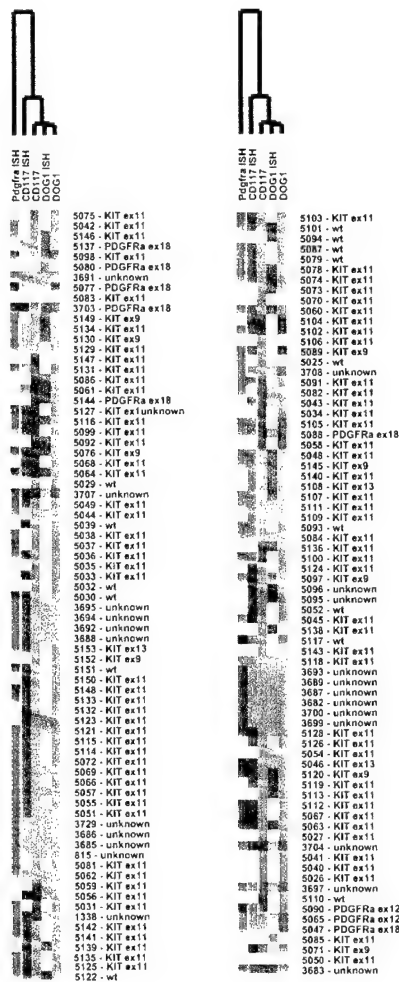


Figure 3

GIST markers by mutation status

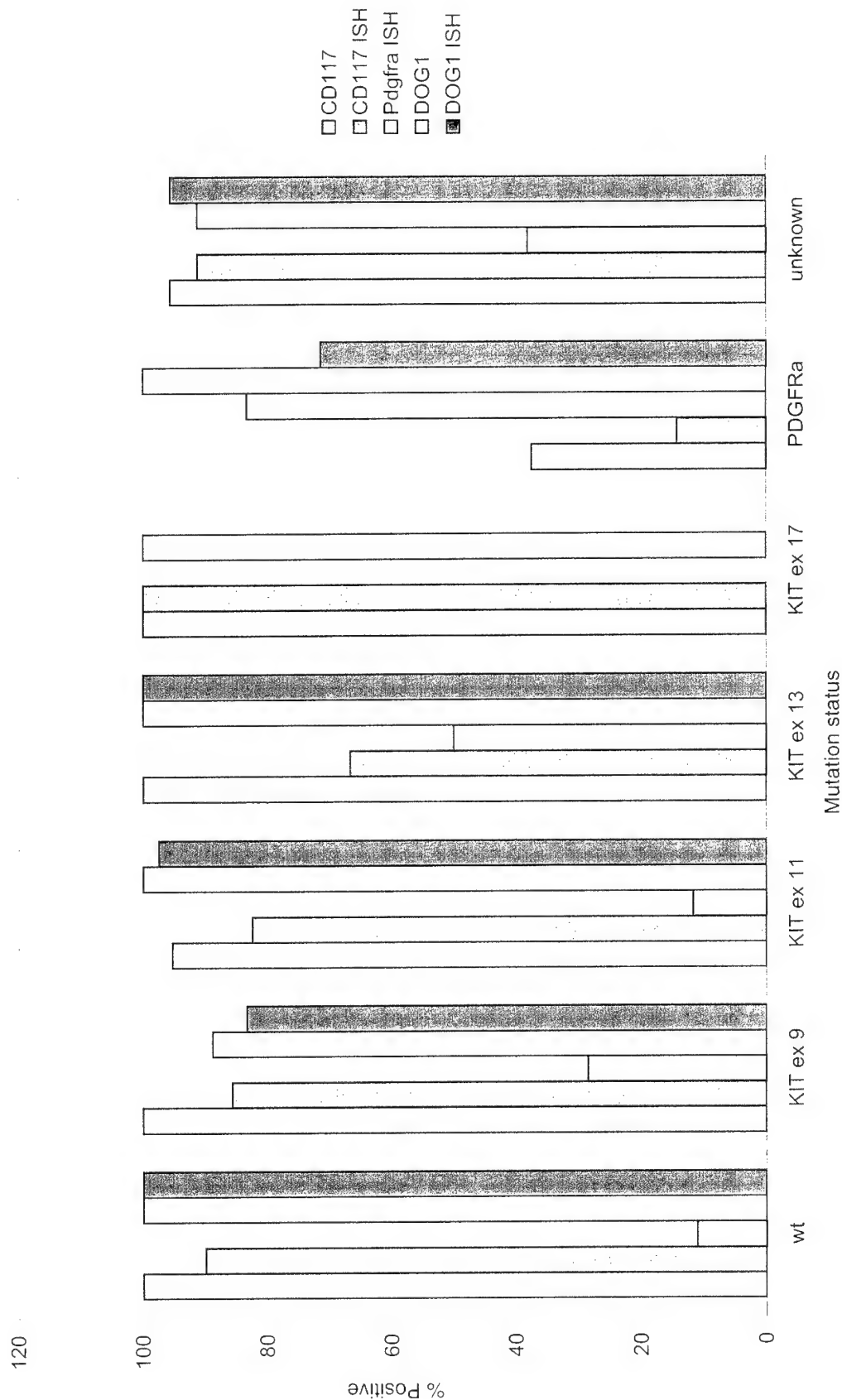


Figure 4

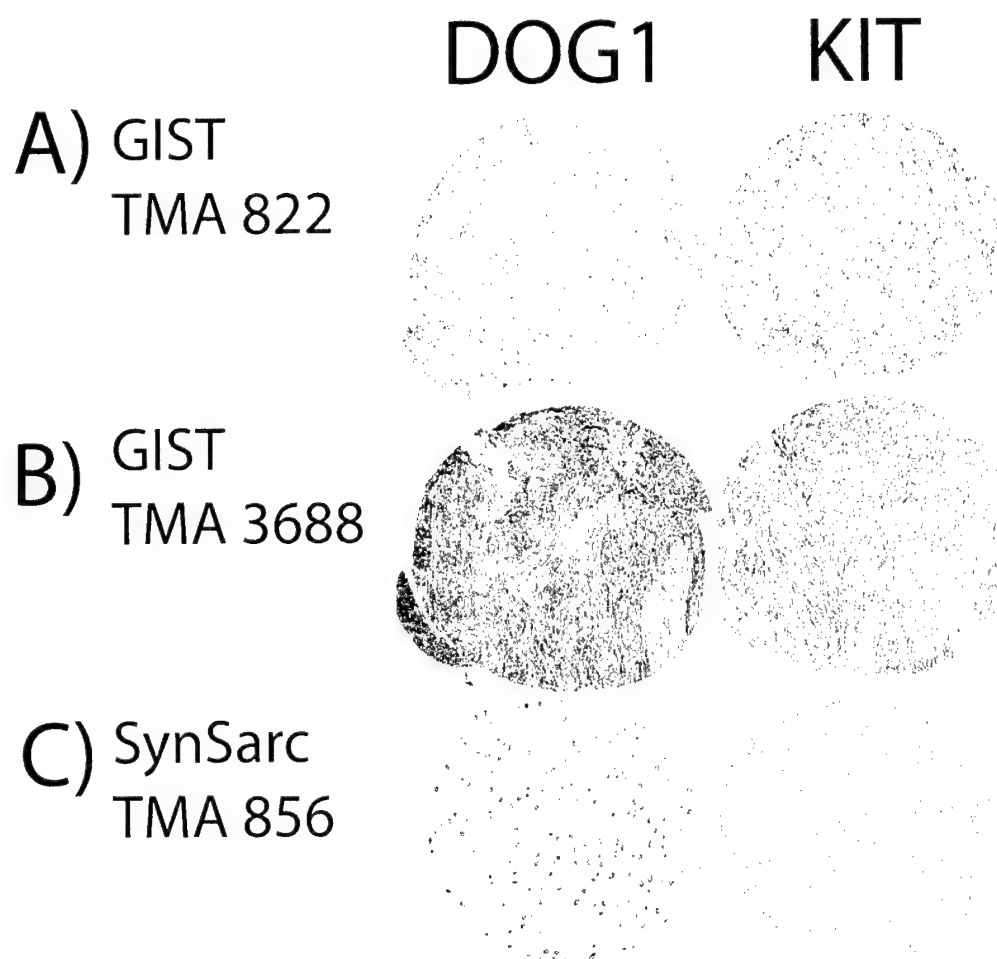


Figure 5

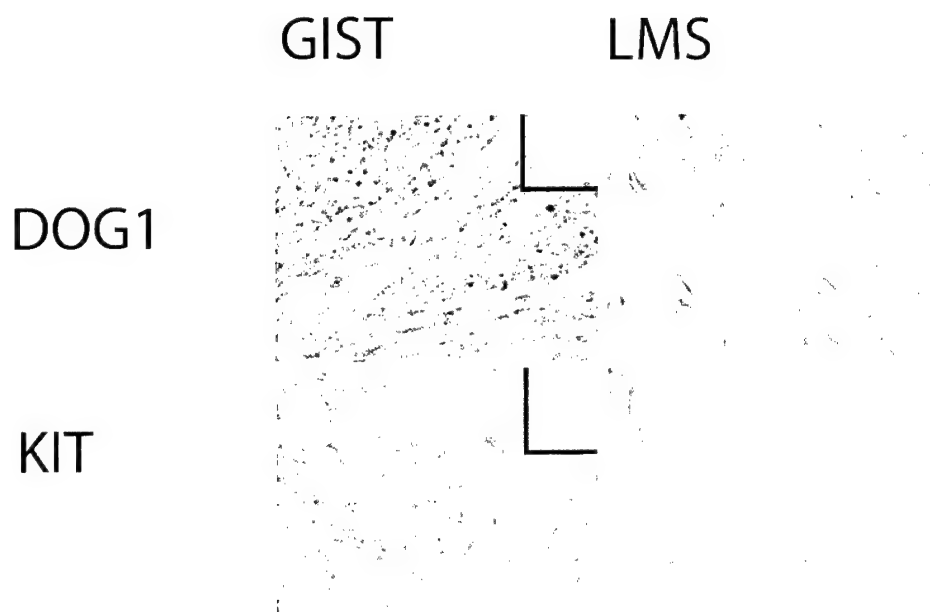
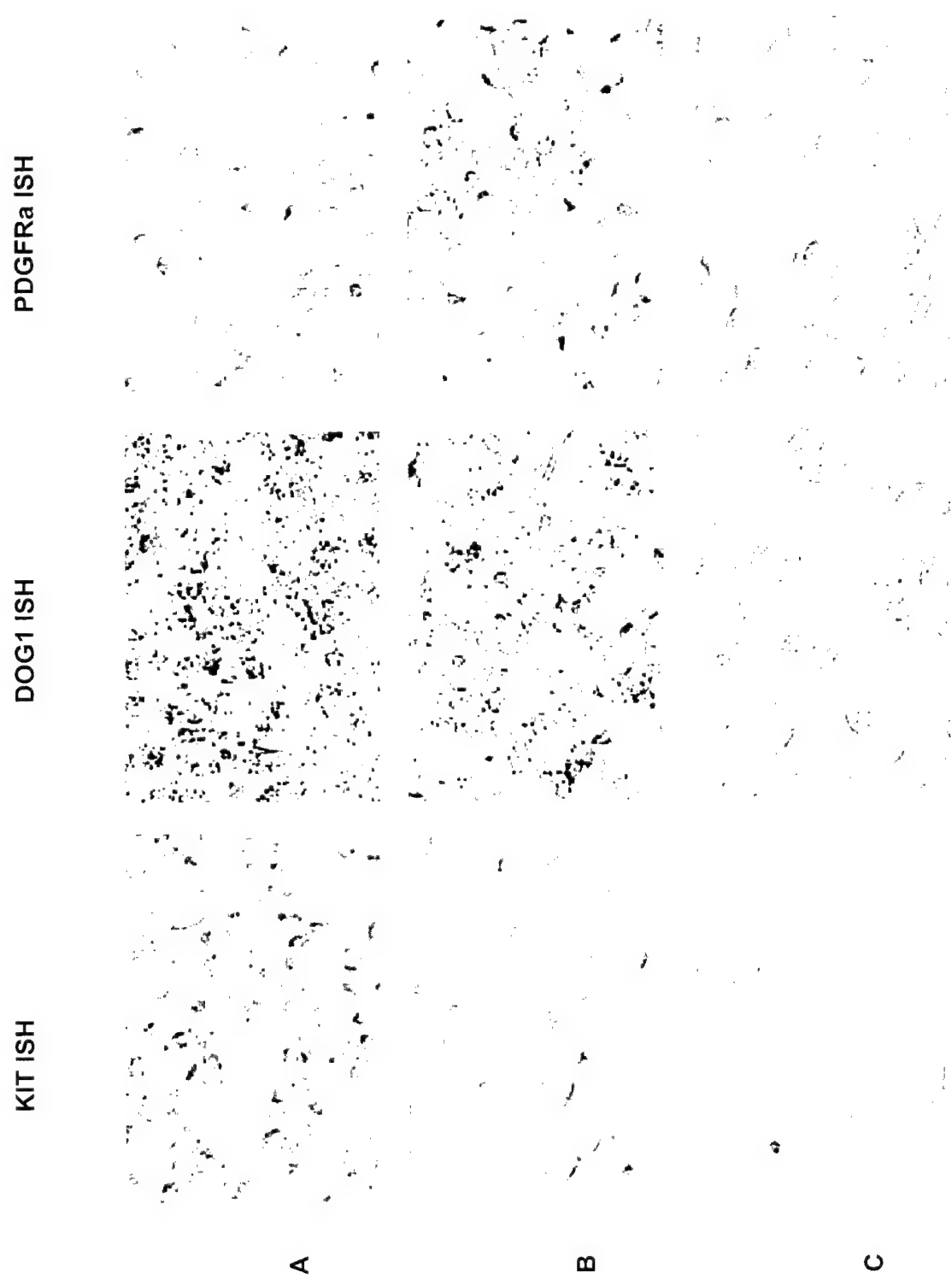


Figure 6



CURRICULUM VITAE**Matt van de Rijn, M.D., Ph.D.****PERSONAL DATA**

Name: Jan Matthijs van de Rijn
Present address: 141 Rocky Creek Road, La Honda, CA 94020
Citizenship: The Netherlands
Visa status: Resident Alien
Medical licensure: State of California: A48323, 6/90
State of Pennsylvania: MD 055043-L, 8/95
Board certification: Anatomic Pathology, 11/93

EDUCATION AND APPOINTMENTS

1974-1979: Medical student at the University of Amsterdam
Received Master's degree in Medicine

1/80-1/82: Graduate student at The Netherlands Cancer Institute
Amsterdam. Dr. Jo Hilgers, adviser

1/82-6/84: Visiting graduate student at the Dana-Farber Cancer Institute
Harvard Medical School, Boston. Dr. Cox Terhorst, adviser

12/84: Received Ph.D., University of Amsterdam
Dr. Piet Borst, thesis supervisor
Field of study: Protein chemistry of T lymphocyte cell
surface antigens

1/85-10/86: Clinical rotations at the Academic Medical Hospital
University of Amsterdam

7/86: Passed ECFMG (Educational Commission for Foreign Medical
Graduates) exam

10/86: Received M.D., University of Amsterdam

11/86-2/89: Postdoctoral fellowship in the laboratory of Dr. I. L. Weissman
Department of Pathology, Stanford University
First year funded by a grant from The Netherlands
Organization for the Advancement of Pure Research

From November 87 funded by a postdoctoral grant from
the

Multiple Sclerosis Society
Field of study: Protein chemistry and genetic analysis of
lymphocyte homing receptors

2/89-6/91: Residency in pathology at Stanford University Medical Center

7/91-6/92: Fellowship in surgical pathology, Department of Pathology
Stanford University Medical Center

Co-chief resident in surgical pathology,
Department of Pathology, Stanford University Medical Center

7/92-6/93: Fellowship in immunopathology, Department of Pathology
Stanford University Medical Center

7/93-6/94: Clinical Fellow, Department of Pathology
Stanford University Medical Center

7/94-6/95: Clinical Assistant Professor, Department of Pathology
Stanford University Medical Center

7/95-6/98: Assistant Professor, Department of Pathology and Laboratory
Medicine, University of Pennsylvania Medical Center

7/98-6/2001: Assistant Professor, Department of Pathology
Stanford University Medical Center.

7/2001-present Associate Professor, Department of Pathology
Stanford University Medical Center.

MEMBERSHIPS

United States and Canadian Academy of Pathology
South Bay Pathology Society
American Society for Investigative Pathology
American Association for Cancer Research

Ad hoc referee for:

American Journal of Clinical Pathology
American Journal of Pathology
Blood
Medical and Pediatric Oncology
Southern Medical Journal
The Journal of Histochemistry and Cytochemistry
Cancer

Modern Pathology
British Journal of Cancer
Cell Biochemistry and Biophysics
The Lancet
New England Journal of Medicine
Clinical Cancer Research

NIH/NCI GRANT REVIEWS, WORKING GROUPS, NATIONAL PANELS

Reviewer for RFA: "Technologies for gene expression in the nervous system"
July 29-30, 1999, Washington, D.C.

Participant and presenter at NCI "Working Group Meeting on Tissue Arrays."
December 16-17, 1999, Rockville, MD.

Participant NCI "State of the Science Sarcoma" meeting June 17-18, 2002, Bethesda, MA

Participant and co-chair of breakout session at NCI "Sarcoma Progress Review Group
Roundtable Meeting". October 8-10, 2003, Philadelphia, PA

Member site-visit committee at Erasmus Postgraduate School of Molecular Medicine,
November 12-14, 2003, Rotterdam, The Netherlands.

EDITORIAL BOARDS

2003	PLOS: Public Library of Science
2004	The American Journal of Pathology

COURSES/MEETINGS

1. The use of molecular biology and immunohistochemistry in the differential diagnosis of soft tissue tumors (with Fred G. Barr, M.D., Ph.D.), April 1998, American Society of Clinical Pathologists, Los Angeles, CA.
2. The use of molecular biology and immunohistochemistry in the differential diagnosis of soft tissue tumors (with Fred G. Barr, M.D., Ph.D.), April 2000, American Society of Clinical Pathologists, Boston, MA.
3. Co-director and speaker at the 2002 Special Course for Advanced Molecular Pathology at the 91st annual meeting of the United States and Canadian Academy of Pathology, Chicago, IL.
4. Co-chair Preferred Papers section: Bone and Soft Tissue Tumors at the 93rd annual meeting of the United States and Canadian Academy of Pathology, Vancouver, Canada.

5. Co-director of the 2004 Special Course for Advanced Molecular Pathology at the 93rd annual meeting of the United States and Canadian Academy of Pathology, Vancouver, Canada.

INVITED LECTURES

1. EBV positive lymphomas. The Netherlands Cancer Institute, October 1994, Amsterdam, The Netherlands.
2. Use of anti-CD34 antibodies in surgical pathology. January 1995, Systemics Inc., Palo Alto, CA.
3. Use of CD34 in immunohistochemistry. Current Concepts in Surgical Pathology, September 1995, Stanford University, Stanford, CA.
4. Mechanisms of molecular techniques in diagnostic surgical pathology. Current concepts in surgical pathology, September 1995, Stanford University, Stanford, CA.
5. The diagnosis and molecular analysis of soft tissue tumors. (with Fred Barr, M.D., Ph.D.) Pathology Society of Philadelphia, October 1996, Philadelphia, PA.
6. Grand Rounds. Chromosomal translocations in soft tissue tumors. Department of Pathology, Cornell University Medical School, March 1998, New York, NY.
7. Chromosomal translocations in small round cell tumors. Department of Pathology, University of Leiden Medical School, April 1998, The Netherlands.
8. Gene microarray analysis on human breast carcinoma. The Netherlands Cancer Institute, May 1999, Amsterdam, The Netherlands.
9. Poorly differentiated synovial sarcoma. Current Issues in Anatomic Pathology. UCSF-Stanford University, May 1999, San Francisco, CA.
10. Chairman of session "Functional Genomics" and talk "Western blot analysis and RNA expression" at Advances in Laser Capture Microdissection, June 1999, NIH, Bethesda, MD.
11. Keynote address. Towards genomic scale analysis of gene expression in human cancer. European Meeting on Molecular Diagnostics, October 15, 1999, Scheveningen, The Netherlands.
12. Towards genomic scale analysis of gene expression in human cancer. Department of Pathology, University of Pennsylvania, December 14, 1999, Philadelphia, PA.
13. Combining laser capture microdissection and tissue arrays with gene microarray studies. Eos Biotechnology, February 24, 2000, San Francisco, CA.

14. Combining laser capture microdissection and tissue arrays with gene microarray studies. Research Genetics, May 22, 2000, Huntsville, AL.
15. Combining laser capture microdissection and tissue arrays with gene microarray studies. Smith-Kline Beecham Laboratories, May 23, 2000, King of Prussia, PA.
16. Study of human tumors using cDNA and tissue microarrays. Vanderbilt University, November 2000, Nashville, TN.
17. Study of human tumors using cDNA and tissue microarrays. University of British Columbia, December 2000, Vancouver, BC.
18. Study of human tumors using cDNA and tissue microarrays. DNAX, Research Institute, January 2001, Palo Alto, CA.
19. Study of soft tissue tumors and lymphomas using cDNA and tissue microarrays. Colorado Society of Pathology, March 22, 2001, Denver, CO.
20. Studying breast carcinoma using cDNA arrays and tissue arrays. Current Issues in Anatomic Pathology. UCSF-Stanford University, May 24, 2001, San Francisco, CA.
21. Cluster analysis of tissue microarray data. Workshop Tissue Microarray Infostructure. Automated Information Management in the Clinical Laboratory Symposium. Ann Arbor, MI, May 30, 2001.
22. Study of human tumors using cDNA and tissue microarrays. Genentech, San Francisco, CA, June 18, 2001.
23. Study of breast carcinoma and sarcomas using cDNA and tissue microarrays. Cottage Hospital, Santa Barbara, CA, September 20, 2001.
24. Studying human sarcomas using gene and tissue microarrays. University of Washington, Seattle, WA, October 5, 2001.
25. Keynote Address. Studying human neoplasms using cDNA and tissue microarrays. 6th Annual Molecular Medicine Day. Rotterdam, The Netherlands, December 14, 2001.
26. Plenary Lecture. Combining gene array and tissue microarray analysis on human tumors. Symposium "Tumor Profiling", American Association of Cancer Research. San Francisco, May 9, 2002.
27. Plenary Lecture: Expression profiling of tumors. 63rd Annual meeting, Society of Investigative Dermatology. Los Angeles, May 16, 2002.

28. Studying soft tissue tumors with gene and tissue microarrays. Current Issues in Anatomic Pathology. UCSF-Stanford University, May 24, 2002, San Francisco, CA.
29. Understanding breast carcinoma using the tools of molecular biology. Breast Pathology: Current concepts and controversies. June 4, 2002, Harvard Medical School, Boston, MA.
30. Data management for tissue microarrays. October 4, 2002, John Radcliffe Hospital, Oxford University, Great Britain.
31. Expression profiling of sarcomas. October 9, 2002, 24th International Congress of the International Academy of Pathology, Amsterdam, The Netherlands.
32. Keynote Address: The use of gene arrays and tissue microarrays for the study of human tumours. October 9, 2002, 24th International Congress of the International Academy of Pathology, Amsterdam, The Netherlands.
33. The use of gene expression arrays and high density tissue arrays in the study of lymphoid malignancies. October 10, 2002, 24th International Congress of the International Academy of Pathology, Amsterdam, The Netherlands.
34. Studying sarcomas with gene expression profiling and tissue microarrays. October 11, 2002, Leiden University Medical School, Leiden, The Netherlands.
35. Studying sarcomas with high throughput techniques. South-Western Oncology Group (SWOG) Meeting. October 28, 2002, San Antonio, TX.
36. Gene Expression Profiling and Comparative Genomic Hybridization Studies on Sarcomas Using Microarrays. October 31, 2002, Queens University, Kingston, Ontario, Canada.
37. Using tissue microarrays in high throughput studies. Director's Challenge meeting, NCI. November 7, 2002, Bethesda, MA.
38. Hardware and software used in gene microarray analysis. 7th ADNAT meeting, CCMB Research Center, March 1, 2003, Hyderabad, India.
39. Tissue microarrays. 7th ADNAT meeting, CCMB Research Center, March 3, 2003, Hyderabad, India.
40. A demonstration of the Stanford Microarray Database. 7th ADNAT meeting, CCMB Research Center, March 6, 2003, Hyderabad, India.
41. High throughput studies on sarcomas. 7th ADNAT meeting, CCMB Research Center, March 7, 2003, Hyderabad, India.

42. Array based comparative genomic hybridization and laser capture microdissection. 7th ADNAT meeting, CCMB Research Center, March 8, 2003, Hyderabad, India.
43. Expression profiling, comparative genomic hybridization and tissue microarray studies on sarcomas. Dept of Pathology, Virginia Commonwealth University, March 21, 2003, Richmond, VA.
44. Study of human soft tissue tumors using cDNA and tissue microarrays. FASEB, American Society for Investigative Pathology. April 12, 2003, San Diego, CA.
45. Studying breast carcinoma using the tools of molecular biology. Breast Pathology: Current concepts and controversies. June 2, 2003, Harvard Medical School, Boston, MA.
46. Application of microarray technology in diagnostic pathology (with Blake Gilks, MD). 54th Annual Meeting of the Canadian Association of Pathologists. July 8, 2003, Charlottetown, Prince Edward Island, Canada.
47. Gene expression arrays in GIST. 3rd international symposium on GIST. September 19, 2003, Helsinki, Finland.
48. Gene and tissue array studies on human tumors. 2nd Ankara Biotechnology Days. September 22, 2003, Ankara, Turkey.
49. Gene microarrays to profile human tumors. BayChem 2003, Am. Ass. for Clin. Chem. October 3, 2003, Redwood City, CA.
50. Molecular profiles of sarcomas. Southwest Oncology Group. October 4, 2003, Seattle, WA.
51. Data analysis for tissue microarrays. October 20, 2003, Centro Nacional de Investigaciones Oncologicas (CNIO), Madrid, Spain.
52. Gene and tissue microarray studies on sarcomas. October 22, 2003, Hospital Sant Pau, Barcelona, Spain.
53. Grand Rounds. Gene and tissue microarray studies on sarcomas. November 3, 2003, Department of Pathology, Brigham and Women's Hospital, Boston, MA
54. EGFR expression measured by gene arrays and tissue microarrays in synovial sarcoma. November 10, 2003, University Hospital Leuven, Belgium.
55. Some examples of extension and validation of gene array data by tissue microarray analysis. November 22, 2003, NCI Director's Challenge Meeting, Bethesda, MA.

56. Grand Rounds. Gene and tissue microarray studies on sarcomas. January 28, 2004. Department of Pathology, UCLA Medical Center, Los Angeles, CA.
57. Gene expression profiling of soft tissue tumors. March 7 2004. International Society of Bone and Soft Tissue Pathology at the 93rd Annual meeting United States and Canadian Academy of Pathology, Vancouver, Canada.
58. Grand Rounds. Gene and tissue microarray studies on sarcomas. March 25, 2004. Department of Pathology, University of North Carolina, Chapel Hill, NC.
59. Applications of gene and tissue microarrays to the diagnosis and classification of sarcomas. March 31, 2004. Sarcoma Diagnosis and Treatment Session at the 95th annual meeting of the American Association of Cancer Research. Orlando, FL.
60. Applications of gene and tissue microarrays to pathology. May 6, 2004. Leopoldina Meeting: Pathologie im Wandel. Rotach-Egern, Germany.

LECTURES at STANFORD UNIVERSITY MEDICAL CENTER

1. Laser capture microdissection and gene microarray analysis. Department of Medicine, February 23, 1999.
2. Gene microarray analysis of human breast carcinomas and laser capture microdissection. Surgery grand rounds, with S. Jeffrey and C. Barry, March 24, 1999.
3. Cancer profiling. Lecture in Biomedical Genomics course, org. Bob Shafer, March 6, 2002.
4. Gene arrays. Medical student talk, Pathology 230 course. April 16, 2003
5. Use of cDNA and tissue microarrays for studies on human tumors. Lecture in Biomedical Genomics course, org. Bob Shafer, February 4, 2004.

PUBLICATIONS

PEER REVIEWED PUBLICATIONS

1. M. van de Rijn, A.H.M. Geurts van Kessel, V. Kroezen, A.J. van Agthoven, K. Versteijnen, C. Terhorst, and J. Hilgers. (1983) Localization of a gene controlling the expression of the human transferrin receptor to the region q12-qter of chromosome 3. *Cytogenetics and Cell Genetics* 36:525-31.

2. M. van de Rijn, P.G. Lerch, R.W. Knowles and C. Terhorst. (1983) The thymic differentiation antigens T6 and M241 are two unusual MHC Class I antigens. *Journal of Immunology* **131**:851-5.
3. P.G. Lerch, M. van de Rijn, P. Schrier and C. Terhorst. (1983) Biochemical comparison of the T6 antigen and the HLA-A,B antigens. *Human Immunology* **6**:13-30.
4. C. Bernabeu, D. Finlay, M. van de Rijn, R.T. Maziarz, P.A. Biro, H. Spits, J.E. de Vries and C. Terhorst. (1983) Expression of the major histocompatibility antigens HLA-A2 and HLA-B7 by DNA mediated gene transfer. *Journal of Immunology* **131**:2032-7.
5. H.J. van der Reijden, E.R. van Wering, M. van de Rijn, C.J.M. Melief, M.B. van't Veer, H. Behrendt and A.E.G.Kr. von dem Borne. (1983) Immunological typing of acute lymphoblastic leukemia. *Scandinavian Journal of Haematology* **30**:356.
6. J.J.M. van Dongen, H. Hooijkaas, K. Hahlen, K. Benne, W.M. Bitter, A.A. van de Linde-Preesman, I.L.M. Tettero, M. van de Rijn, J. Hilgers, G.E. van Zanen and A. Hagemeijer. (1984) Detection of minimal residual disease in Tdt positive T-cell malignancies by double immunofluorescence staining. In: Minimal Residual Disease in Acute Leukemia. (B. Lowenberg, and A. Hagebeek, eds.) Martinus Nijhoff Publishers b. v., The Hague, The Netherlands.
7. M. van de Rijn, P.G. Lerch, B.R. Bronstein, R.W. Knowles, A.K. Bhan and C. Terhorst. (1984) Human cutaneous dendritic cells express two glycoproteins T6 and M241 which are biochemically identical to those found on cortical thymocytes. *Human Immunology* **9**:201-10.
8. C. Bernabeu, M. van de Rijn, P.G. Lerch and C. Terhorst. (1984) β 2-microglobulin from serum associates with MHC Class I antigens on the surface of cultured cells. *Nature* **308**:642-5.
9. M. van de Rijn, C. Bernabeu, B. Royer-Pokora, J. Leiss, J.G. Seidman, H. Spits, J. E. de Vries and C. Terhorst. (1984) Recognition of HLA-A2 by cytotoxic T-lymphocytes after DNA transfer into human and murine cells. *Science* **226**:1083-5.
10. P. Snow, M. van de Rijn and C. Terhorst. (1985) Association of the T8 T-cell differentiation antigen with the Class I MHC thymocyte antigen T6 on the thymocyte cell surface. *European Journal of Immunology* **15**:529-32.
11. P.G. Lerch, M. van de Rijn, J.E. Smart, R.W. Knowles and C. Terhorst. (1985) Isolation and purification of the human thymocyte antigens T6 and M241. *Molecular Immunology* **23**:131-9.

12. H. Spits, W. van Schooten, H. Keizer, G. van Seventer, M. van de Rijn, C. Terhorst and J.E. de Vries. (1986) Evidence that recognition of alloantigen by the T-cell receptor of allospecific cytotoxic T-cell clones (CTL) has to be preceded by antigen nonspecific conjugate formation between the CTL clone and target cells. *Science* **232**:403-5.
13. M. Siegelman, M. van de Rijn, and I.L. Weissman. (1989) Mouse lymph node homing receptor cDNA clone encodes a glycoprotein revealing tandem interaction domains. *Science* **243**:1165-72.
14. M. van de Rijn, S. Heimfeld, G.J. Spangrude, and I.L. Weissman. (1989) Mouse hematopoietic stem cell antigen Sca-1 is a member of the Ly-6 antigen family. *Proceedings of the National Academy of Sciences* **86**:4634-8.
15. M. van de Rijn, I.L. Weissman, and M. Siegelman. (1990) Biosynthesis pathway of gp90^{MEL-14}, the mouse lymph node-specific homing receptor. *Journal of Immunology* **145**:1477-82.
16. M. van de Rijn, D.P. Regula, Jr., and M. Billingham. (1990) Autopsy findings after coronary rotational atherectomy. *American Journal of Cardiovascular Pathology* **3**:301-4.
17. O. Kamel, M. van de Rijn, L. Weiss, G. Del Zoppo, P. Hench, B. Robbins, P. Montgomery, R. Warnke, R. Dorfman. (1993) Reversible lymphomas associated with Epstein-Barr virus occurring during methotrexate therapy for rheumatoid arthritis and dermatomyositis. *New England Journal of Medicine* **328**:1317-21.
18. M. van de Rijn, M.R. Hendrickson, R.V. Rouse. (1994) An immunohistochemical study of inflammatory fibroid polyps of the gastrointestinal tract. *Applied Immunohistochemistry* **2**:54-9.
19. B.R. Smoller, M. van de Rijn, D. LeBrun, R.A. Warnke. (1994) *bcl-2* expression reliably distinguishes trichoepitheliomas from basal cell carcinomas. *British Journal of Dermatology* **131**:28-31.
20. M. van de Rijn, C.M. Lombard, R.V. Rouse. (1994) Expression of CD34 by solitary fibrous tumors of the pleura, mediastinum and lung. *American Journal of Surgical Pathology* **18**:814-20.
21. M.N. Rizeq, M. van de Rijn, M.R. Hendrickson, R.V. Rouse. (1994) A comparative immunohistochemical study of uterine smooth muscle neoplasms with emphasis on the epithelioid variant. *Human Pathology* **25**:671-77.
22. M. van de Rijn, M.R. Hendrickson, R.V. Rouse. (1994) CD34 expression by gastrointestinal tract stromal tumors. *Human Pathology* **25**:766-71.

23. O.W. Kamel, M. van de Rijn, D.P. LeBrun, L.M. Weiss, R.A. Warnke, R.F. Dorfman. (1994) Lymphoid neoplasms in patients with rheumatoid arthritis and dermatomyositis: frequency of Epstein-Barr virus and other features associated with immunosuppression. *Human Pathology* **25**:638-43.
24. V. Bhargava, D. Kell, M. van de Rijn, R.A. Warnke. (1994) *bcl-2* immunoreactivity in breast carcinoma correlates with hormone receptor positivity. *American Journal of Pathology* **145**:535-40.
25. A.B. Gelb, M. van de Rijn, D.P. Regula, J.P. Cornbleet, O.W. Kamel, D.S. Horoupian, M.L. Cleary, R.A. Warnke. (1994) Epstein-Barr virus associated natural killer-large granular lymphocyte leukemia. *Human Pathology* **25**:953-60.
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SUBMITTED

1. C.B. Gilks, B.C. Vanderhyden, S. Zhu, M. van de Rijn, T.A. Longacre. Distinction between serous tumors of low malignant potential and serous carcinomas based on global mRNA expression profiling.
2. F.P. Li, J.A. Fletcher, M. Heinrich, J.E. Garber, S.E. Sallan, S. Higham, C. Curiel-Lewandrowski, A. Duensing, M. van de Rijn, L.E. Schnipper, G.D. Demetri. Familial gastrointestinal stromal tumor syndrome (GISTs): phenotypic and molecular features in a kindred.

IN PREPARATION

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